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Maturation of Initially Infected *Hymenolepis nana* Under Rapid Protective Immunity Against Reinfection with the Eggs

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Introduction

In the mouse-*Hymenolepis nana* system, initial oncospheral invasion into the mouse intestinal villi elicits protective immunity against subsequent egg challenge within 2 days (Hearin, 1941; Heyneman, 1962) and even a single oncosphere in the tissue is sufficient to make the host immune (Ito and Yamamoto, 1976). Nevertheless the highly immunogenic oncospheres which acted as immunogens continue to develop as "normally" to tailless, mouse-derived cysticercoids (cysts) in 4–5 days (Ito, 1977a) and further to mature tapeworms in about 14 days in the same immunized host (Hunninen, 1935). This may also be supported by the following fact that the lumen phase established by inoculating as many as 2,000 mouse-derived cysts does not make the host immune during the prepatent period (Ito *et al.*, 1977).

However, there is no direct experimental evidence that the time course of the initially established worms' development is normal. It is unknown whether or not the growth rate, or maturation rate of established worms, alleged to be normal, is influenced by the rapid immunity and is the results of the host responses or the results of "parasitism" (Brown, 1976).

In the present study we counted the number of gravid proglottids of all tapeworms recovered from (1) egg-inoculated and mouse-derived cyst-inoculated mice, and (2) immunosuppressed and normal mice, both of them given eggs.

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Materials and Methods

Parasites

The preparation of shell-free eggs or mouse-derived cysticercoids of *H. nana* has been previously described (Ito, 1975; 1977a). Eggs were collected from egg donors killed 2–7 weeks after initial inoculation with 100 shell-free eggs/mouse and mouse-derived cysticercoids from cysticercoid donors killed 5 days post initial inoculation with 20,000 or more shell-free eggs/mouse. Throughout the text, the word "cyst" means mouse-derived cysticercoid recovered from the mouse intestine, and the word "egg" means shell-free egg, except where specifically stated to be otherwise.

Animals

Worm-free, 5±1 week old mice of both sexes of dd strain (random-bred) were used for all experiments. Worm-free mice were multiplied from worm-free closed colony and have been kept on bedding in plastic cages in clean room in this laboratory; for several years, no eggs of metazoan parasites have been found at any time examined. Every litter was divided into two groups and kept on bedding in separate plastic cages from day 0 (the day of egg inoculation). All the

cages were kept clean by renewing the bedding (White Flake, Nippon Charls River Co. Ltd., Japan) every day except Sunday and the cage itself once a week. Mice inoculated with eggs (on day 0) or cysts (on day 5) were housed in separate room. Diet (NMF, Oriental Yeast Co. Ltd., Japan) and water were available *ad libitum*.

Experimental Design

Experiment I. Twelve litters were used; 2, 4, 4 and 2 litters were examined on days 10, 12, 14 and 16, respectively. One mouse from every litter consisted of 8–12 mice was used as cyst donor, and remainders of the same litter were randomly divided into two groups. One was egg group given 100 eggs/mouse on day 0 and another was cyst group given 100 cysts/mouse on day 5. These groups of the same litter were killed on the same day.

On day 0 cyst donor(s) and each of egg group mice were given 20,000 or more eggs and 100 eggs, respectively. On day 5 cysts were collected from cyst donor(s) by Ito's method (1977a) and 100 cysts suspended in 0.1–0.2 ml 0.9% NaCl solution were directly administered into the mouse stomach of cyst group(s) by a glass pipette under light ether anesthesia (Ito *et al.*, 1977).

Experiment II. One litter was examined on day 10, five litters on day 12 and three litters on day 14. Every litter was randomly divided into two groups. One group (cortisone group) was pretreated with cortisone acetate (2 mg/day/mouse) just before inoculation with 100 eggs, another nontreated group (NT group) was given 100 eggs of the same batch with no pretreatment. Cortisone acetate (Cortone, 25 mg/ml, Nippon Merk-Banyu Co. Ltd., Japan, adjusted so that 1 mg was suspended in 0.1 ml sterile 0.9% NaCl solution) was administered twice on days −1 and 0 (2 hr prior to egg inoculation) by dosal subcutaneous injection.

For proving the immunosuppressive effect of this cortisone dose, four litters of 32 mice were examined. They were randomly divided into two groups; one was cortisone group, another was NT group. All the mice were given 100 eggs/mouse on day 0, challenged with 2,000 eggs on day 10, killed on day 14 and initially established tapeworms in the lumen and secondary established cysts in the villi were counted (Ito, 1975).

Criterion for the maturity of tapeworms

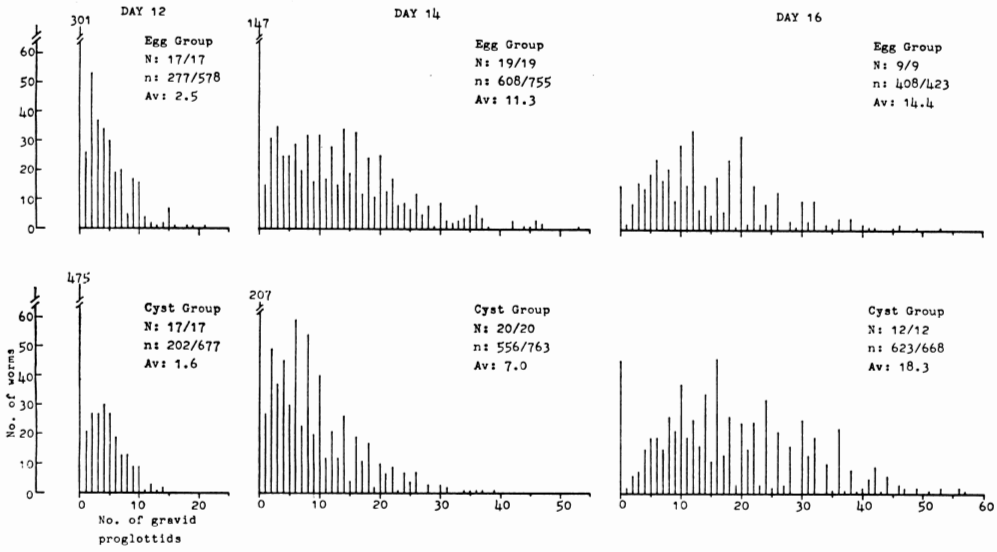
On day 10, 12, 14 or 16, all the mice were killed at the corresponding time ±1 hr to the definite time on day 0. Each small intestine was removed, cut open lengthwise very carefully not to damage tapeworms dwelling in the intestine (Ito, 1975; Sawada, 1955) and shaken vigorously in fresh tap water at room temperature in a 15 cm diameter petri dish. Then all tapeworms recovered from each mouse were placed in fresh tap water in an 8 cm diameter petri dish and kept at room temperature for 30–60 min. Soon after the relaxed tapeworms were placed on glass slide with just enough tap water to cover the worms, the number of gravid proglottids was counted under ×100 or ×200 magnification, and the length of each worm was measured. In the text, the word "gravid proglottids" means proglottids harboring infective eggs exclusively (Moriyama, 1961). Average number of gravid proglottids of the populations (Av) recovered on day 12, 14 or 16 was estimated by the following formula.

$$\mathrm{Av}=\frac{\text{Total No. of gravid proglottids}}{\text{Total No. of worms recovered}}$$

Results

Experiment I. Comparison of worm maturity between egg and cyst groups. On every day 12, 14 or 16, the results of maturation rates of worms recovered from egg and cyst groups are illustrated in Fig. 1. The number of mice infected, the number of worms recovered, and the average number of gravid proglottids are all recorded in Fig. 1.

On day 10 about 400 worms were recovered from either egg or cyst group consisted of 10 mice each, but no worms had gravid proglottids and the results were, therefore, excluded from Fig. 1, whereas on days 12, 14 and 16, mature worms were found as



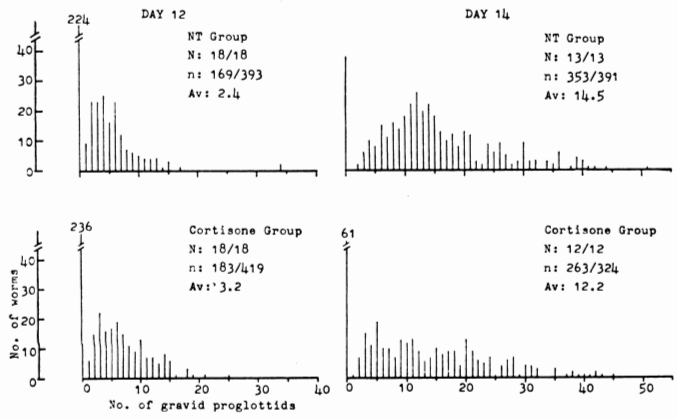


Fig. 1 Maturation rates of *Hymenolepis nana* of the same age recovered from egg- and cyst-inoculated mice.
N: No. of mice found infected/ No. of mice inoculated, n: No. of mature worms/ No. of worms recovered, Av: Average No. of gravid proglottids.

Fig. 2 Maturation rates of *H. nana* of the same age recovered from cortisone injected and non-treated mice, both of them given eggs.
N, n, Av: *see* legends of Fig. 1.

shown in Fig. 1. Among the litters in which the size (8–12 mice) or the ages (4–6 weeks old) were somewhat different, maturation rate was also somewhat different, but little difference in the same litter when the size of worm population (population density) of each litter-mate was similar to one another. Further in a population recovered from each mouse of egg or cyst group, there appeared no difference of either maturation rates or lengths of the worms; the worms in a population established by inoculating eggs or cysts appeared to develop synchronously, at least by day 16.

In either group gravid proglottid formation started between 10 and 12 days post egg inoculation and most of worms became mature within 16 days. In some litters, within 14 days, almost all of worms did become mature. There was a tendency that the more the worms recovered, the less gravid proglottids formed in each worm.

Experiment II. Comparison of worm maturity between immunosuppressed and normal mice. Maturation rates of worms recovered from either cortisone group or NT group on days 12 and 14 are summarized in Fig. 2. That the cortisone dose (4 mg/mouse) was sufficient to make the host of 5±1 weeks old immunosuppressive was proven by induction of

reinfection : fourteen of 17 mice of cortisone group survived until the day of autopsy (day 14) and 29.7±12.8 (S.D.) tapeworms were recovered and 11 of the 14 mice harbored 148.3±125.1 cysts in the villi simultaneously, whereas 15 mice of NT group harbored 32±16.8 tapeworms but no cysts.

On day 10, about 250 worms were recovered from either group of 5 mice each, but all of them were immature, and the result was excluded from Fig. 2. There was no difference of maturation rates between the two groups on days 10, 12 and 14, although the two groups should be clearly different from each other in the presence or absence of protective immunity against *H. nana*.

Discussion

The present results strongly suggest that maturation of initially infected *H. nana* is not influenced by the rapid protective immunity against reinfection and the growth rate is normal at least within 16 days.

Experiment I was carried out to compare maturation rates of worms recovered from mice given eggs or cysts. This is, as far as we know, the first examination comparing the dual (tissue and lumen) phases of *H. nana* in the mouse directly without interference of the intermediate host, such as *Tribolium confusum* (*c.f.* Ghazal and Avery, 1974). Comparative study of maturation rates by the use of eggs and tailed, beetle-derived cysts recovered from the beetle intermediate hosts (Ghazal and Avery, 1974) is very interesting in itself for recognizing the difference of the features of direct and indirect cycles of this parasite (Anderson, 1976), but this is not adequate to recognize the dual phases in the direct cycle (Ito *et al.*, 1977). From this point of view it seems very questionable to speculate that the difference of growth or maturation rates of worms recovered from mice inoculated with eggs or beetle-derived cysts, if exists, is attributable to the presence or absence of immunity acquired within 2 days of egg inoculation (Ghazal and Avery, 1974). Because there is no proof that beetle-derived cysts are antigenically and/or physiologically equivalent to mouse-derived cysts, and it seems rather unlikely (Caley, 1975 ; Lackie, 1976).

At present we believe that the beetle-derived cyst should not be utilized instead of the mouse-derived cyst for studying the problem of the direct cycle. These ambiguities by the use of beetle-derived cysts described above are excludable by the use of eggs and mouse-derived cysts originated from the same batch of eggs. We found no difference of maturation rates between these two groups from day 10 to day 16 ; during the earlier days of the dwarf tapeworm, at least. Nevertheless, from this results, we could not exclude the following two ambiguities. That the cysts recovered from the mouse intestine might have been affected by the rapid immunity is the first, and the cysts inoculated may, therefore, act in the same manner in the lumen as in egg-inoculated hosts, although tissue phase with the inoculated worms could not exist in cyst-inoculated hosts ; the time course of maturation might have been completely set out during the preceding tissue phase. The second is whether or not the maturation rate of cyst-derived tapeworms is somewhat delayed. In cyst donors a number of eggs was inoculated, therefore, cysts recovered from cyst donors might be influenced by the so called "crowding effect" and might be smaller or younger than those recovered from egg group mice inoculated with 100 eggs/mouse. However, these possibilities seem not to be found, because in the former the time course of cyst development is not affected by the rapid immunity (Ito *et al.*, 1977) and in the latter egg dose does not affect the size or the age of cysts isolated from intestines (unpublished).

These ambiguities should be excluded from experimental results by inoculating the same dose of eggs into the immunosuppressed and normal mice as demonstrated in experiment II. Cortisone suppresses protective immunity against egg challenge (Okamoto, 1969) : the cortisone dose used in experiment II was sufficient to suppress the protective immunity.

Although there was a remarkable difference of susceptibility to reinfection between cortisone and NT groups, maturation rates between these two groups were not distinguishable.

From these two experimental results it seems probable to consider that maturation of initially infected *H. nana* of the direct cycle is not influenced by the immunity against reinfection at least by day 16. The development into mature tapeworms seems to be normal even in the immunized host by day 16 and the lumen phase of *H. nana* appears to be different from oncosphere in its immunogenicity (Ito, 1977 b ; Ito *et al.*, 1977) as speculated by Brown (1976). This is very interesting for considering the mechanism of the worm survival in the immunized host, although we did not follow the maturation rates after day 16, because too many gravid proglottids to be counted accurately. We have therefore been undertaking other experiments pursuing maturation rates during the full time of worm survival by counting total egg production of each population in the intestines (but not in feaces) and similar results have been obtained (unpublished).

The life span of *H. nana* in mice is generally conceived to be very short (Hunninen, 1935), however, a small number of egg-derived *H. nana* can often survive at least 6–10 months in some of the immunized mice (Ito, in preparation), and we have no information as to whether this short life span is the results of immunological expulsion as proven in other lumen dwelling *Hymenolepis* infections (Befus, 1975 ; Hopkins and Stallard, 1974 ; Hopkins and Zajac, 1976). At present we should not exploit easily the expulsion system proven in other species of *Hymenolepis* for explanation of the short life span of *H. nana* in the definitive, mouse host, although it seems likely, because biological features of *H. nana* in the mouse seem to be unique to this species and might be quite different from other *Hymenolepis* of indirect cycle. Further works should be done before discussing this problem.

Summary

Maturation rates of initially established tapeworms of *Hymenolepis nana* were measured from day 10 to day 16 by counting the number of gravid proglottids of all tapeworms recovered from (1) egg-inoculated and mouse-derived cysticercoid-inoculated mice, and (2) immunosuppressed and normal mice, both of them given eggs. Within day 16 maturation of the worms was not influenced by the rapid protective immunity, which was acquired in mice within 2 days of initial egg inoculation, against reinfection with eggs. This result suggests that initially established worms, early stage(s) of which should have stimulated a strong protective immunity, develop normally in the same immunized host and that the lumen phase of initially infected *H. nana* of direct cycle is different from oncosphere in its immunogenicity.

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再感染防御下での小形条虫の成熟速度

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マウス小腸に寄生している小形条虫の全虫体について受胎節数を顕微鏡下で数える方法により，虫卵投与日の10日後から16日目，或いは14日目までの期間での初感染虫体の成熟速度を調べた．虫卵投与後2日以内で獲得される再感染防御免疫が免疫原として働いた初感染虫体の成熟速度に影響を及ぼすか否かについて次の2つの実験系を通して考察を加えた．

実験Ⅰ．虫卵投与（免疫）マウスと無尾擬嚢尾虫投与（非免疫）マウスとでの成虫の成熟速度の比較

実験Ⅱ．コーチゾン注射（免疫抑制）マウスと未処置（免疫）マウスとでの虫卵由来成虫の成熟速度の比較

両実験において計21腹の5±1週令マウスを使用した．同腹個体を上記いずれかの2群に無作意に分け，同一日時に剖検し，成虫の成熟速度を比較した結果，免疫獲得の有無による明確な相違があつたにもかかわらず，虫体の成熟速度に差は認められなかつた．

この結果は小形条虫の無尾擬嚢尾虫（マウス小腸絨毛内発育型）は六鉤幼虫とは免疫原性を異にすることを強く示唆する．このことは免疫宿主体内での小形条虫初感染虫体の生存を可能ならしめる一因を成すものかも知れない．